Future Foods 9 (2024) 100307

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo

Effect of oil on the rheological properties and reaction behavior of highly concentrated wheat gluten under conditions relevant to high moisture extrusion

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ARTICLE INFO

Keywords:
Wheat gluten
High moisture extrusion
Meat analog
Oil droplets
Closed cavity rheometer
CLSM

ABSTRACT

Understanding the rheological behavior of highly concentrated proteins is critical for the development of meat analogs. To enhance their sensory appeal, plant oil is often added during extrusion. The objective of this study was to gain a better understanding of how oil affects the rheological properties of gluten. Gluten doughs with different oil contents (0% to 6%) were prepared by adding emulsified and non-emulsified oil. Rheological measurements were performed under extrusion-relevant conditions using a closed cavity rheometer. In addition, the oil phase was visualized by CLSM imaging before and after rheological measurements. It was found that the complex modulus $|G^*|$ of the wheat gluten decreased with increasing oil concentration. When oil was added as emulsion, $|G^*|$ was significantly higher compared to when non-emulsified oil was added. The CLSM images revealed that the oil droplet size increased with increasing oil concentration. The doughs with emulsified oil had significantly smaller oil droplets. The results suggest that oil droplets act as fillers, weakening the protein matrix. By reducing the oil droplet size, this weakening effect could be reduced. Therefore, we concluded that the oil droplet size is a critical parameter influencing the rheological properties of oil-containing wheat gluten doughs under extrusion-relevant conditions.

1. Introduction

Plant-based meat analogs have become increasingly popular in recent years. The reasons for this are diverse, including animal welfare, health reasons, and environmental concerns. A widely used technology for the production of such meat analogs is high moisture extrusion (water content 40–80%). In this process, plant proteins are mixed with water, heated, sheared by the rotation of the screws, and finally forced through a cooling die, where the fibrous, anisotropic structure of the product is created, which imitates the muscle fibers of real meat (Högg and Rauh, 2023; Palanisamy et al., 2019; van der Sman and van der Goot, 2023; Wittek et al., 2021c). However, a major challenge with such meat analogs are their poor sensory properties, especially in terms of tenderness and juiciness. For real meat, these sensory characteristics are strongly influenced by intramuscular fat. To improve the low sensory appeal of meat analogs, oil or fat can be added during the extrusion (Egbert and Borders, 2006; Wang et al., 2022b). However, the addition of even small amounts of oil during extrusion processing leads to significant undesired changes in the final product properties, e.g. less pronounced fibrous and meat-like texture (Opaluwa et al., 2023; Wang et al., 2023). In the extrusion process, the rheological properties of the material play a crucial role, as they have a great influence on the process conditions as well as on the formation of the final product properties, such as the fibrousness (Pietsch et al., 2019a; Wittek et al., 2021a). The addition of oil during extrusion usually leads to a reduction in viscosity. The effect of oil on the viscosity can be described by a “plasticizing effect” or a “filler effect”, which are both described in the next two paragraphs.

According to Ilo et al. (2000), oil exerts a “plasticizing effect” on macromolecules by reducing the friction between them. They describe that triglycerides in the same way as water lead to an increase in the molecular mobility of the macromolecules resulting in a decrease in viscosity. Guy (1994) refers to the effect of oil on macromolecules as a lubricant. They emphasize that the effect of oil as a lubricant is much

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https://doi.org/10.1016/j.fufo.2024.100307
Received 31 August 2023; Received in revised form 14 December 2023; Accepted 23 January 2024
Available online 26 January 2024

greater in terms of its active concentration than the effect of water, which acts as plasticizer. Generally, plasticizers are defined as relatively small molecules that are incorporated into a polymeric material, like proteins, to make it more flexible. In order to explain the mechanism of plasticizers, the lubricity theory, the gel theory and the free volume theory are described in the literature (Di Gioia and Guilbert, 1999; Hernandez-Izquierdo and Krochta, 2008; Rowat et al., 2021). According to the lubricity theory, plasticizers facilitate the motion of the entangled protein molecules past one another, thus reducing molecular friction by acting like a lubricant. The gel theory extends the lubricity theory as it assumes that the plasticizer acts by disrupting non-covalent protein--protein interactions (such as hydrogen bonds, van der Waals interactions, and electrostatic forces) and by masking these binding sites from each other, thereby preventing their re-aggregation. At the same time, unattached plasticizer molecules increase the free volume of the protein molecule, giving it its flexibility. Combining free volume theory with gel and lubricity theory implies that plasticizer molecules, which do not interact with the protein chain, fill up the free volume created by the interacting molecules (Mekonnen et al., 2013). For a plasticizer to be effective in increasing the protein flexibility, it needs to be compatible with the protein molecules. Thus, it needs to consist of groups that have an affinity to the protein ("protein-philic") as well as groups that have no affinity to the protein ("protein-phobic"). Protein-philic groups allow for an effective penetration of the plasticizer into the protein, while protein-phobic groups are more effective in disrupting protein-protein interactions in order to increase the free volume. Due to the increase in free volume and the reduction in entanglement density, the addition of a plasticizer generally results in a viscosity reduction of the protein matrix (Lefèvre et al., 2005; Rowat et al., 2021). Molecules typically found in food fats and oils such as mono-, di-, triglycerides or free fatty acids could be molecularly incorporated into the protein matrix and act as plasticizers due to their small size.

During extrusion processing, the oil phase is known to be dispersed into droplets in the aqueous, highly concentrated protein matrix (Gwiazda et al., 1987; Kendler et al., 2021). From emulsion-filled food gels, e.g. yoghurt, cheese or mayonnaise, which are defined as polymeric gels of proteins or polysaccharides in which emulsion droplets are embedded as "fillers", it is known that oil droplets have a great influence on the mechanical and rheological properties of the matrix. Important influencing parameters are the oil concentration, the oil droplet size or the oil-matrix interactions, which mainly depend on the surfactants used to stabilize the oil droplets (van Vliet, 1988). When the oil droplets interact with the protein matrix, they are referred to as active fillers, while droplets that do not interact with the protein matrix are referred to as inactive fillers (Chen and Dickinson, 1999; McClements et al., 1993). However, both the storage modulus of the protein matrix and the filler itself have a major influence on whether the filler leads to reinforcement or weakening of the protein network (Dille et al., 2015; Farjami and Madadlou, 2019; Geremias-Andrade et al., 2016). In the case of meat analogs, the modulus of the continuous protein matrix is much higher than the modulus of the dispersed oil droplets. Therefore, irrespective of whether the oil droplets are bound or unbound, both have the same effect on the modulus of the matrix, namely a decrease with increasing oil concentration (Sala et al., 2007).

During extrusion processing, proteins are plasticized with water and heated and sheared at the same time, which leads to structural changes in the protein molecules. These changes in molecular structure include protein unfolding and protein cross-linking reactions via covalent and non-covalent bonds, and have a significant impact on the final product properties of meat analogs (Akdogan, 1999; Arêas, 1992; Liu and Hsieh, 2008). Wheat gluten which is used in this study polymerizes via disulfide bonds upon thermal and mechanical treatment, resulting in the formation of a three-dimensional viscoelastic protein network (Chen et al., 2020; Jia et al., 2020; Pietsch et al., 2019b; Pommet et al., 2004). Polymerization of wheat gluten leads to changes in its rheological properties. Emin et al. (2017) studied the polymerization behavior of gluten under extrusion-like conditions by monitoring the changes in rheological properties with a closed cavity rheometer. They observed that the temperature and shear rate as well as the water content have a great influence on the polymerization reactions of wheat gluten. However, information on how the addition of oil to wheat gluten plasticized with water affects the polymerization behavior as well as the rheological properties of the protein network cannot be found in literature.

The aim of this study is therefore to systematically investigate the influence of oil on the rheological properties of wheat gluten under defined conditions. In particular, we looked at conditions that are relevant to high moisture extrusion applications. We hypothesize that the size of the oil droplets has a greater impact on the rheological properties of the wheat gluten network, than the properties, e.g., chemical structure or purity, of the oil itself. To evaluate the underlying hypothesis, samples with different oil concentrations, oil droplet size distributions and oil types were prepared and investigated for their rheological behavior under extrusion-relevant conditions.

2. Materials and methods

2.1. Materials

Vital wheat gluten was kindly provided by Kröner Stärke (Ibbenbüren, Germany). According to the manufacturer's specifications, it contains max. 8% moisture and 83% protein on a dry matter basis. Medium-chain triglyceride oil (MCT oil, WITARIX®60/40) purchased from IOI Oleo GmbH (Hamburg, Germany) is referred to as "oil" throughout this paper. According to the manufacturer's specification the MCT oil consists of 60% caprylic acid (C8:0) and 40% capric acid (C10:0). Refined rapeseed oil was purchased from B. Schell GmbH (Lichtenau, Germany) and is referred to as "rapeseed oil (standard)". According to the manufacturer's specification, the main fatty acids of the rapeseed oil triglycerides are oleic acid (C18:1) with 52–65%, linoleic acid (C18:2) with 19–30% and α-linolenic acid (C18:3) with 8–12%. Moreover, the rapeseed oil can contain up to 0.5% free fatty acids according to manufacturer's specification. To remove impurities (e.g. free fatty acids, mono- and diglycerides) from the rapeseed oil, it was additionally purified with florisil® (Carl Roth, Karlsruhe, Germany) according to Dopierala et al. (2011). Purified rapeseed oil is referred to as "rapeseed oil (purified)". The interfacial tension against water was determined for all three oil types with the pendant drop method (OCA 15 LJ, DataPhysics Instruments GmbH, Filderstadt, Germany) at constant temperature of 23 °C for at least 100 min according to Leister and Karbstein (2021). For the emulsification, Tween20® supplied by Carl Roth (Karlsruhe, Germany) was used. For CLSM imaging, all oils were stained at a concentration of 0.02 g/l with the lipophilic fluorescent dye nile red (Carl Roth, Karlsruhe, Germany) before dough mixing.

2.2. Dough preparation

Gluten doughs were mixed using a lab-scale co-rotating twin screw extruder (Process11, ThermoFisher Scientific Inc., Waltham, MA, USA) operated at room temperature. To avoid extensive heating due to friction during dough mixing, a screw configuration consisting of only forward transport elements and a screw speed of 500 rpm was used. Doughs with two different methods of oil addition (non-emulsified/emulsified) were prepared. For both doughs, the final composition is the same. The composition of the doughs was calculated in such a way that the water and gluten content was reduced to the same extent as the oil content was increased from 0 to 6 wt%, resulting in a constant water-protein ratio for all doughs. The composition of the doughs is summarized in Table 1.

First, doughs with "non-emulsified oil" were prepared. Therefore, Tween20 was dissolved in water and oil was stained with nile red prior to dough mixing. For dough mixing, the gluten was fed into the first barrel, Tween20-water was pumped into the third barrel and oil was

Table 1
Composition of the gluten doughs.

Sample name	Oil content /%	Water content /%	Gluten content /%	Tween20 content /%
0% oil	0	38.8	59.9	1.3
0% oil + 6% water	0	42.4	56.3	1.3
2% oil (non-emulsified)	2.0	38.0	58.7	1.3
2% oil (emulsified)	2.0	38.0	58.7	1.3
4% oil (non-emulsified)	4.0	37.2	57.5	1.3
4% oil (emulsified)	4.0	37.2	57.5	1.3
6% oil (non-emulsified)	6.0	36.4	56.3	1.3
6% oil (emulsified)	6.0	36.4	56.3	1.3

pumped into the fourth barrel of the extruder. Through the rotation of the screws the ingredients were mixed thoroughly so that homogeneous doughs were obtained at the end of the extruder. The doughs with non-emulsified oil were prepared for MCT oil only.

Secondly, doughs with “emulsified oil” were prepared from oil-in-water emulsions with different oil contents. Here, the oil stained with nile red was dispersed in the continuous water phase in which Tween20 was dissolved using an Ultra-Turrax T25 (IKA Werke GmbH & Co. KG, Staufen, Germany) at a rotor speed of 5200 rpm for 5 min. Subsequently, the coarse emulsions were further homogenized in a Microfluidizer MF 110 EH (Microfluidics Corporation, Newton, MA, USA) at 900 bar in one step. Oil-in-water emulsions were prepared for three different oil types, i.e. MCT oil, rapeseed oil (standard) and rapeseed oil (purified). Emulsions were prepared the day before dough mixing and stored overnight at 4 °C. For the dough mixing, gluten was fed into the first barrel of the extruder and then the oil-in-water emulsions were pumped into the third barrel of the extruder. In addition to the doughs with “non-emulsified oil” and “emulsified oil”, another dough was prepared in which the amount of 6 wt% oil was replaced 1:1 by an additional 6 wt% water. This dough is referred to as “0% oil + 6% water” throughout this paper. The composition of all doughs used for this study are depicted in Table 1. After dough mixing, the doughs were vacuumed, frozen, and stored at −18 °C until further use.

2.3. Rheological measurements

The rheological properties of the doughs were determined by an oscillatory closed cavity rheometer (CCR) (RPA elite, TA Instruments, New Castle, DE, USA). The doughs were thawed at room temperature before measurement. 5.5 g of the dough were placed between the two cones of the cavity. To prevent water loss during heating, the cavity is sealed and pressurized with 4.5 bar. The two cones are temperature-controlled and grooved in order to prevent slippage of the material. During measurement the upper cone remains stationary, while the lower cone oscillates in a strain-controlled mode. From the resulting torque response, rheological properties such as the complex modulus are calculated. The function of the closed cavity rheometer is described in more detail by Wittek et al. (2020).

2.3.1. Temperature sweep

The influence of temperature was investigated by heating the doughs at a constant heating rate of 5 K/min from 40 to 180 °C, applying a strain of 0.98% and a frequency of 1.0 Hz, which equals a shear rate of 0.06 s^{-1}. For all doughs, the selected frequency and strain were within the linear viscoelastic (LVE) region, ensuring that the material was not irreversibly changed by mechanical stress. All temperature sweeps were carried out in triplicate.

2.3.2. Time sweep

In order to obtain more detailed information on the time-dependent polymerization behavior of gluten as a function of different oil concentrations and types, isothermal time sweep analyses were carried out. The influence of both, thermal treatment and thermomechanical treatment, which is typical for extrusion applications, were analyzed independently. To investigate the effect of thermal treatment, the doughs were treated at 120 °C for 30 min, using a frequency of 1.0 Hz and a strain of 0.98%, which is within the linear viscoelastic region (LVE). To analyze the nonlinear viscoelastic material response, the doughs were also subjected to thermomechanical treatment. Hereby, for 30 min a constant temperature of 120 °C and an additional mechanical stress was applied by setting a frequency of 10 Hz and a strain of 80%, corresponding to a shear rate of 50.3 s^{-1}. All time sweeps were measured three times.

2.3.3. Strain sweep

Strain sweeps were carried out to characterize the structural strength of a gluten network formed during simultaneous heating and shearing. For this purpose, doughs were pre-treated for 3 min, corresponding to typical residence times in an extruder, at 120 °C and a shear rate of 50.3 s^{-1} (frequency: 10 Hz, strain: 80%) and then cooled to 65 °C at a heating rate of 15 K/min to prevent further polymerization of the gluten during the strain sweeps. Strain sweeps were then performed at a frequency of 1.0 Hz over a strain range of 0.1–1000%. All strain sweeps were performed in triplicate. We define the value of the stress and the value of the strain at the end of the linear viscoelastic (LVE) region as yield stress and yield strain, respectively.

2.4. Visualization of oil phase via CLSM

Confocal laser scanning microscopy (CLSM) was used to compare the size of oil droplets in the doughs as well as in the pellets taken from the closed cavity rheometer after time sweeps. For this purpose, the frozen doughs were cut into small pieces (20 × 9 × 9 mm) and embedded in FSC 22 Blue Frozen Section Medium (Leica Biosystems GmbH, Nussloch, Germany) at −17 °C. Using a CM 3050 cryo-microtome (Leica Biosystems GmbH, Nussloch, Germany), the pieces were cut into 40 μm thick slices, which were then placed on slides, dried at 55 °C, and fixated to the slides using Mowiol (Carl Roth, Karlsruhe, Germany) and a cover slide. A 63x Plan Apochromat/1.4 oil DIC immersion objective was used to analyze the cryo-microtome slices in the CLSM (LSM 510 META, Carl Zeiss Microscope Systems, Jena, Germany). Therefore, the samples were excited with an argon laser at a wavelength of 488 nm. To distinguish the signal of the stained oil from the signal of the autofluorescent gluten, the emitted light was filtered with two different channels (oil: 575–615 nm, gluten: 420–515 nm). In the CLSM images, the gluten matrix appears in green, while the oil appears in red.

2.5. Statistical analysis

OriginPro 2020 software (version 9.7.) from OriginLab Corporation (Northampton, USA) was used for statistical analysis. Differences between samples containing emulsified and non-emulsified oil, samples with different oil/water contents and samples containing different oil types were evaluated by one-way ANOVA using Scheffé’s test for comparison of means with a probability of $p < 0.05$ to identify significant differences.

3. Results

3.1. Influence of oil on gluten polymerization as a function of temperature monitored by complex modulus

Wheat gluten doughs with 0% oil and with 6% non-emulsified oil were heated at a constant heating rate of 5 K/min from 40 to 180 °C at a low shear rate of 0.06 s^{-1}. Fig. 1(a) shows the complex modulus as a function of temperature. For both samples, the evolution of the complex modulus |G*| is characteristic of wheat gluten and already described in literature (Emin et al., 2017). The curve consists of three distinct regions and provides information about the reaction behavior of gluten. First, the complex modulus decreases to a local minimum at about 70–80 °C. This decrease can be attributed to an increase in molecular mobility. Second, a sharp increase in the complex modulus is observed until a local maximum of |G*| is reached at about 135 °C. This increase is caused by an increase in molecular size as a result of crosslinking reactions. Third, a decrease in the complex modulus is observed. This decrease indicates that the polymerization reactions are largely completed, leading to a decrease in |G*| due to higher molecular mobility as the temperature continues to increase. In addition, there is the possibility that degradation reactions occur at high temperatures, contributing to a decrease in |G*| (Kokini et al., 1994; Pommet et al., 2004).

Comparing the samples with 0% oil and 6% oil, it can be observed that the absolute values of the complex modulus decrease with the addition of oil while the characteristic shape is not remarkably changed. The same was seen for samples containing 2% and 4% oil (data not shown). That the addition of oil reduces the modulus of highly concentrated proteins, has also been reported in previous studies (Kendler et al., 2021; Wang et al., 2022a). However, no difference in the position of the local minima or maxima of |G*| was observed for samples with oil. This indicates that for thermal treatment within the LVE region, the reaction rate of the polymerization reactions is not remarkably affected by oil.

To investigate the time-dependent polymerization behavior of wheat gluten as a function of oil concentration, small-deformation measurements (strain: 0.98%; frequency: 1.0 Hz) at a constant temperature of 120 °C were performed. In Fig. 1(b) the change in complex modulus |G*| over time is depicted for wheat gluten doughs containing 0%, 2%, 4% and 6% non-emulsified oil. For all samples, the complex modulus increases until a maximum is reached after approximately 9.5 min. Further heating has no effect on |G*| as a plateau is reached. This indicates that polymerization reactions have been completed (Emin et al., 2017). The time until reaching the maximum |G*| does not differ significantly ($p > 0.05$) between the samples with and without oil, indicating that the reaction rate of the polymerization reactions is not affected by the oil concentration. As already found in the temperature sweeps, the absolute values of the maximum |G*| decrease with increasing oil concentration (0–6%) from 80 kPa to 50 kPa.

3.2. *Comparison of non-emulsified oil, emulsified oil and water addition with regard to their effects on rheological properties of gluten*

The aim of this chapter is to investigate the effect of oil on the rheological properties and the reaction behavior of wheat gluten in more detail. In the following, two doughs with the same oil concentration of 6 wt% which were produced with different oil addition methods, i.e. non-emulsified and emulsified, will be compared. A comparison is also made with a third dough in which the 6 wt% oil was replaced by 6 wt% additional water, in order to compare the plasticizing effect of oil and water.

3.2.1. *Thermal treatment*

Fig. 2(a) shows the change in complex modulus due to thermal treatment (120 °C) at small deformations (strain: 0.98%; frequency: 1.0 Hz) for doughs with 0% oil, 0% oil + 6% water, 6% non-emulsified oil, and 6% emulsified oil as a function of time. The curves for 0% oil and 6% non-emulsified oil are the same as the curves in Fig. 1(b). For all samples depicted in Fig. 2(a) an initial increase in |G*| can be observed, which is due to the polymerization of gluten molecules. After 9.5 min, a maximum is reached, indicating that the polymerization reactions have been completed. |G*| of the 0% oil dough is significantly higher ($p > 0.05$) than the |G*| of the three doughs containing 6% oil (emulsified/non-emulsified) or 6% additional water. Comparing the three samples 0% oil + 6% water, 6% non-emulsified oil, and 6% emulsified oil, it can be observed that all samples show the same behavior. There are no significant differences ($p > 0.05$) in the maximum values of |G*|. These results indicate that within the linear viscoelastic region (LVE), the difference in the absolute values of |G*| between the 0% oil sample and the three other samples (0% oil + 6% water, 6% non-emulsified oil, and 6% emulsified oil) can be attributed to a "dilution effect" by reducing the concentration of high viscous gluten with low viscous liquid. Moreover, it can be observed that the time to reach the maximum of |G*| does not differ between the three samples. This is in agreement with results from Emin et al. (2017). They reported that only water contents below 30% have a hindering effect on the aggregation of gluten molecules, whereas at water contents above 30%, an increase in water content does not lead to any change in the reaction behavior of wheat gluten.

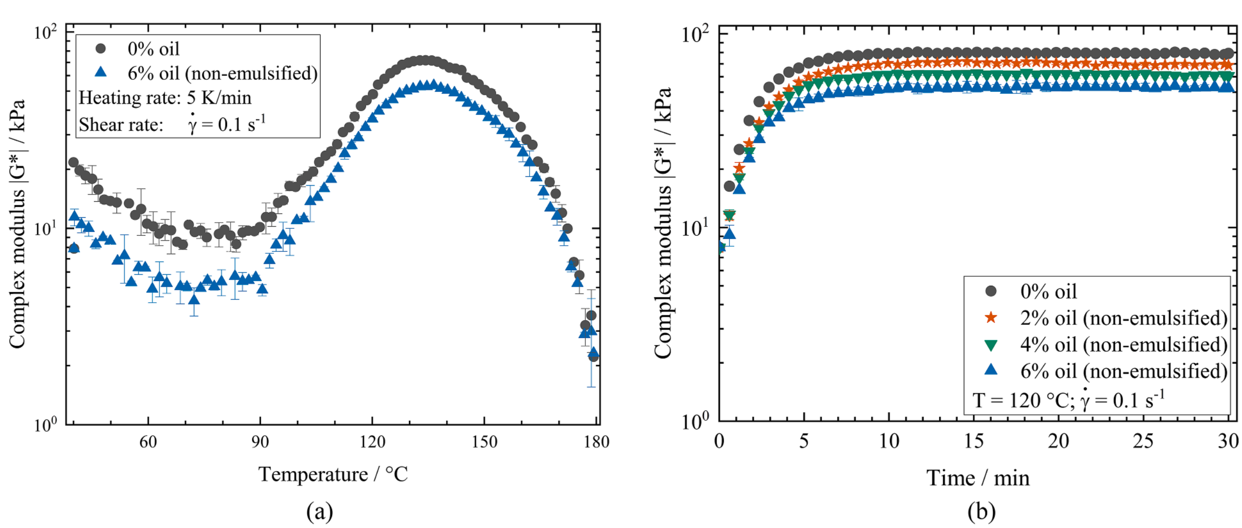

Fig. 1. (a) Complex modulus |G*| as a function of temperature for wheat gluten doughs containing 0% oil and 6% non-emulsified oil. The temperature was increased with a constant heating rate of 5 K/min. The strain and frequency were kept constant at 0.98% and 1.0 Hz, respectively. (b) Change in complex modulus |G*| of wheat gluten as a function of time and different oil concentrations (non-emulsified). The temperature was kept at 120 °C and the strain and frequency were kept constant at 0.98% and 1.0 Hz, respectively.

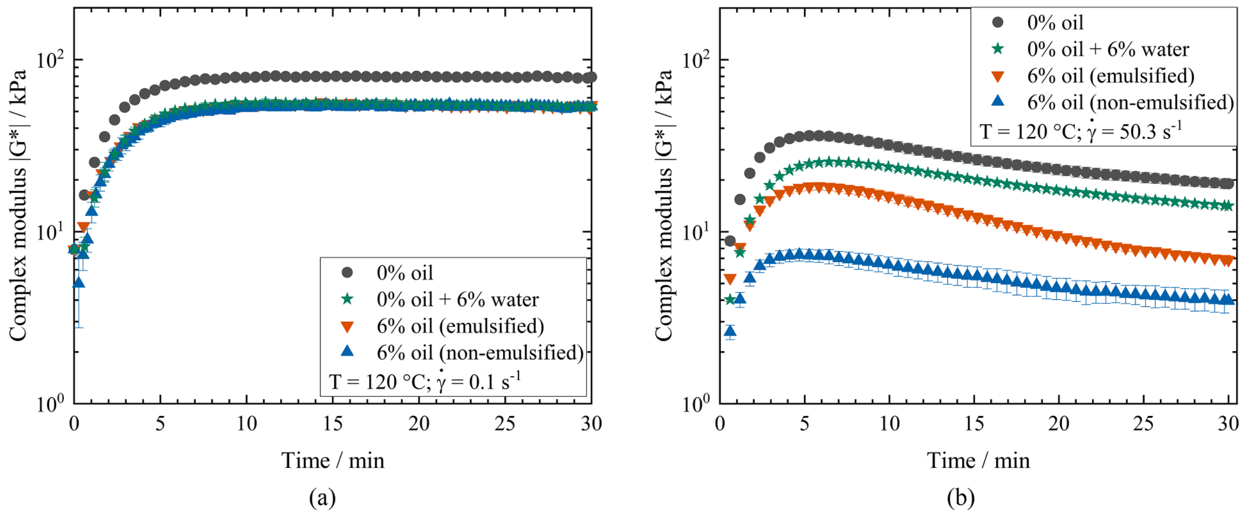

Fig. 2. Change in complex modulus |G*| of wheat gluten doughs containing 0% oil, 0% oil + 6% water, 6% oil (emulsified) and 6% oil (non-emulsified) as a function of time during (a) thermal treatment (temperature: 120 °C; strain: 0.98%; frequency: 1.0 Hz) and (b) thermomechanical treatment (temperature: 120 °C; strain: 80%; frequency: 10 Hz).

3.2.2. *Thermomechanical treatment*

During extrusion, proteins are subjected to both thermal and mechanical stresses. To evaluate the effect of such thermomechanical treatment on the rheological properties of gluten, the doughs were treated at 120 °C and 50.3 s^{-1}, while the complex modulus was detected simultaneously. The change in complex modulus |G*| during thermomechanical treatment is shown in Fig. 2(b) as a function of time. Comparing the both plots from Fig. 2 it can be observed that in Fig. 2(b) the absolute values of |G*| are lower than in Fig. 2(a) for all samples. This is due to a shear-thinning behavior that is typical for highly concentrated proteins. For all doughs depicted in Fig. 2(b) the complex modulus increases initially until a local maximum is reached, followed by a further decrease in |G*|. The increase in |G*| can be attributed to polymerization reactions, while the decrease in |G*| indicates that the degradation of disulfide bonds is taking place. The same rheological changes have been reported in literature for both wheat gluten plasticized with water and wheat gluten plasticized with glycerol (Pietsch et al., 2018; Pommet et al., 2004). The absolute values of the maximum |G*| are significantly different for all samples ($p > 0.05$) and decrease as follows: 36 kPa (0% oil) > 26 kPa (0% oil + 6% water) > 19 kPa (6% oil, emulsified) > 7 kPa (6% oil, non-emulsified).

Comparing the emulsified oil with the non-emulsified samples at all oil concentrations (2%, 4%, 6% oil), it can be seen that the absolute values of |G*| are always significantly higher ($p > 0.05$) for the doughs with emulsified oil (see Appendix Table A1). The largest difference between the doughs with non-emulsified and emulsified oil was found at an oil concentration of 6%. Here, the maximum |G*| of the emulsified oil sample was almost a factor of three higher than the maximum |G*| of the non-emulsified oil sample. Differences between doughs with non-emulsified oil and emulsified oil can also be observed when comparing the time scale of sweeps. While for the doughs with emulsified oil the time to reach the max. |G*| does not change significantly ($p > 0.05$) with increasing oil content, for doughs with non-emulsified oil the time to reach the max. |G*| decreases significantly ($p > 0.05$) from 5.4 min to 3.9 min as the oil content increases from 0% to 6% (see Appendix Table A1). Since significant differences were observed between doughs with non-emulsified and emulsified oil, despite having the same ingredient composition, the results indicate that oil dispersion plays a crucial role in the rheological properties as well as the reaction behavior of wheat gluten.

3.2.3. *Strain-dependency of gluten networks formed under thermomechanical treatment*

In order to compare the deformability and strength of different gluten networks formed by thermomechanical treatment, strain sweeps were performed. Therefore, the four doughs described in the previous Section 3.2.2. (0% oil, 0% oil + 6% water, 6% non-emulsified oil, 6% emulsified oil) were first subjected to thermomechanical treatment, cooled down in order to prevent further crosslinking reactions and finally characterized by strain sweeps. In Fig. 3, the storage modulus G' and the loss modulus G'' of the four doughs are depicted as a function of the strain. It is evident that in the linear viscoelastic (LVE) region, the storage modulus G' is higher than the loss modulus G'' for all samples. This is typical for solid-like, elastic materials with gel-like behavior, such as meat analogs, which consist of highly concentrated protein networks (Schreuders et al., 2021; Wittek et al., 2021b).

Within the LVE range, the applied strain leads to a reversible deformation of the protein network. Therefore, G' shows no dependence on the strain. However, once a critical strain is exceeded, G' decreases significantly and strain thinning occurs. As seen in Fig. 3, the gluten

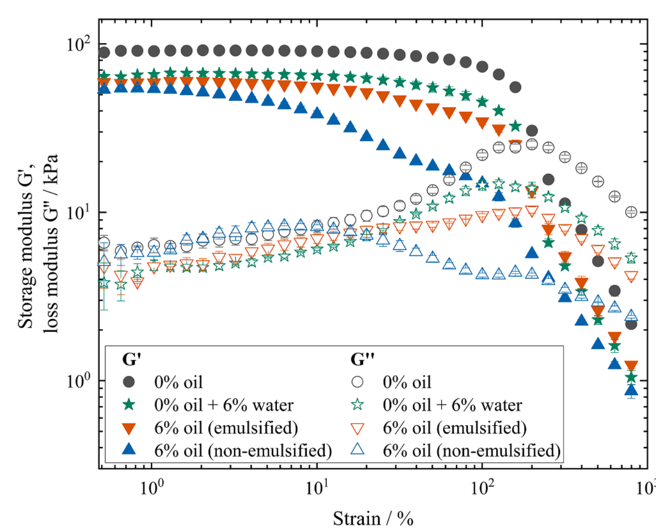

Fig. 3. Strain sweeps of wheat gluten doughs containing 0% oil, 0% oil + 6% water, 6% oil (emulsified) and 6% oil (non-emulsified). The temperature was kept constant at 65 °C and the frequency was set to 1.0 Hz.

network containing 0% oil has a yield strain of 100%, which equals a yield stress of around 70 kPa. By adding oil or water both the yield strain and the yield stress decrease significantly ($p > 0.05$). The network containing 6% non-emulsified oil sustains a yield strain of only 10% and a yield stress of 4 kPa before strain thinning occurs. The maximum strain, defined as yield strain, within the LVE region provides information about the strength of the protein network. Strong and elastic networks can withstand large deformations before irreversible changes occur in the material. Within the LVE region, this is reflected in a higher maximum strain compared to weak networks (Hyun et al., 2011). Thus, the results indicate that the strongest network is formed for the sample with 0% oil, while the weakest network is formed, when 6% non-emulsified oil is added.

Looking at the curves of the loss modulus G'', a local maximum before the crossover point of G' and G'' can be observed. This so-called "weak strain overshoot" is most pronounced for the sample containing 0% oil and is decreasing for the samples containing 0% oil + 6% water, 6% emulsified oil and 6% non-emulsified oil, respectively. The latter almost shows no strain overshoot. The weak strain overshoot phenomena is well known for different soft materials like concentrated emulsions, suspensions or polymer networks (Hyun et al., 2011). For physical polymer networks, Tirtaatmadja et al. (1997) explains that as the stress increases the disruption rate of cross-links is higher than the formation rate leading to a decrease in junction density and an increase in G'' until the network collapses completely. However, Hyun et al. (2011) describes that the structural cause of the strain overshoot in G'' is not universal and material-specific.

For emulsion-filled gels, it is reported that fracture or material failure starts from defects in the protein matrix. Oil droplets can act as such defects and thus function as stress concentration nuclei (also called "structure-breakers") from where fracture starts (Sala et al., 2007). We observed that as the oil concentration and thus the number of oil droplets increases the material fracture starts at lower yield strains, indicating a weakening of the protein network (see Appendix, Figure A1). This is in accordance with results from previous studies (Saavedra Isusi et al., 2023b; Wang et al., 2022a). Comparing the samples 6% non-emulsified oil with 6% emulsified oil, significant differences in the yield strain are observed before material failure occurs. This indicates that the oil droplet size has a strong effect on the protein network strength.

3.3. Comparison of different oil types with regard to their influence on guten polymerization monitored by complex modulus

In order to investigate the impact of the oil type on the rheological behavior of wheat gluten, the measurements of Section 3.2. were additionally applied to wheat gluten doughs containing rapeseed oil. In this chapter, all doughs were prepared from oil-in-water emulsions. Rapeseed oil was chosen due to its different chemical structure compared to MCT oil. While MCT oil is a medium chain triglyceride with only saturated fatty acids, the rapeseed oil is less defined and consists mostly of mono- and polyunsaturated fatty acids. Furthermore, rapeseed oil contains polar impurities (e.g. free fatty acids), which are naturally present in commercial oils. This polar compounds have a great impact on the interfacial tension of the oil against water. In order to investigate if polar impurities and thus changes in interfacial tension have an impact on the rheological behavior, the rapeseed oil was additionally purified with florisil®. For MCT oil, rapeseed oil (standard) and rapeseed oil (purified) the interfacial tensions against water are 24.5 ± 0.1, 15.3 ± 0.2 and 28.9 ± 0.3 mNm^{-1}, respectively. Rapeseed oil (purified) has the highest interfacial tension, followed by the MCT oil and the rapeseed oil (standard). Due to the purification step the interfacial tension of rapeseed oil increased by almost 50% which is in accordance with literature (Leister and Karbstein, 2021).

3.3.1. Thermal treatment

In Fig. 4(a), the complex moduli $|G^*|$ of doughs containing 0% oil and 4% MCT oil, rapeseed oil (standard) and rapeseed oil (purified) are depicted as a function of time during thermal treatment (120 °C, 0.1 s^{-1}). The complex modulus of all samples first increases within the first 5 min until a plateau is reached. The absolute values of $|G^*|$ of the dough with 0% oil is higher than of the doughs containing 4% oil, which is consistent with the results of Section 3.2.1. Between the doughs containing different oil types, there is no significant difference ($p > 0.05$). These results indicate that despite having different interfacial tensions ranging from 15.3 to 28.9 mNm^{-1}, the different types of oil used do not affect the rheological behavior of wheat gluten in the linear viscoelastic (LVE) region.

3.3.2. Thermomechanical treatment

To simulate extrusion-like conditions, the doughs containing different oil types were subjected to thermomechanical treatment (120 °C, 50.3 s^{-1}). In Fig. 4(b), the change in complex modulus $|G^*|$ is depicted as a function of time for doughs containing 0% oil, 4% MCT oil, 4% rapeseed oil (standard) and 4% rapeseed oil (purified). The curve of the dough with 0% oil is the same with the curve in Section 3.2.2. and is only shown for comparison. For all samples, an increase in $|G^*|$ is observed in the first 5 min, followed by a steep decrease in $|G^*|$. The complex modulus $|G^*|$ of the dough with 0% oil is significantly higher ($p > 0.05$) than $|G^*|$ of the doughs containing 4% oil. These results are in agreement with the results from Section 3.2.2.

Comparing the doughs with different oil types, no significant difference ($p > 0.05$) between the curves can be seen until approximately 15 min are reached. The maximum $|G^*|$ are 25.9 kPa, 23.5 kPa and 24.9 kPa for 4% MCT oil, 4% rapeseed oil (standard) and rapeseed oil (purified), respectively. The times of reaching maximum $|G^*|$ are 5.3 min, 5.8 min and 5.5 min for 4% MCT oil, 4% rapeseed oil (standard) and rapeseed oil (purified), respectively. The same trend can be observed for doughs containing 2% and 6% oil (see Appendix, Table A2). After 15 min, the decrease in $|G^*|$ of the dough containing 4% MCT oil is slightly less pronounced than the decrease in $|G^*|$ of both doughs containing 4% rapeseed oil. In summary, the results show that the effect of the different oil types on the rheological behavior of wheat gluten doughs during thermomechanical treatment in the time range relevant for extrusion processing (a few minutes) is not significant.

3.3.3. Strain-dependency of gluten networks formed under thermomechanical treatment

Strain sweeps were performed to determine whether the oil type had an effect on the wheat gluten network formed during thermomechanical treatment (120 °C, 50.3 s^{-1}, 3 min). Fig. 5 shows the storage modulus G' and the loss modulus G'' as a function of strain. The curve of the dough with 0% oil is identical to the curve in Section 3.2.3 and is only shown for comparison. It can be seen that the addition of 4% MCT shortens the length of the LVE region from a strain of about 100% (dough with 0% oil) to 80% (4% MCT oil). This indicates that the gluten network with 4% MCT oil is weaker and less elastic than the gluten network without oil. This observation is consistent with the results in Section 3.2.3. The plots of the doughs with rapeseed oil are slightly different from the plots of the doughs with MCT oil. While the samples with 4% MCT oil show a clearly pronounced plateau of G' up to a strain of 80% before strain thinning occurs, the samples with 4% rapeseed oil show a less pronounced plateau. Once a strain of 15% is exceeded, G' of the rapeseed samples decreases with a small slope, until a strain of 80% is reached. Once the strain exceeds 80%, strain thinning can be observed, leading to complete structural failure. When comparing the two doughs with rapeseed oil (standard) and rapeseed oil (purified), no difference can be found. Both plots overlap each other, which is in line with the observations made in the previous sections. Moreover, the "weak strain overshoot" of the loss modulus G'' is less pronounced, when 4% oil is added. While for the 4% MCT oil sample a small weak strain overshoot

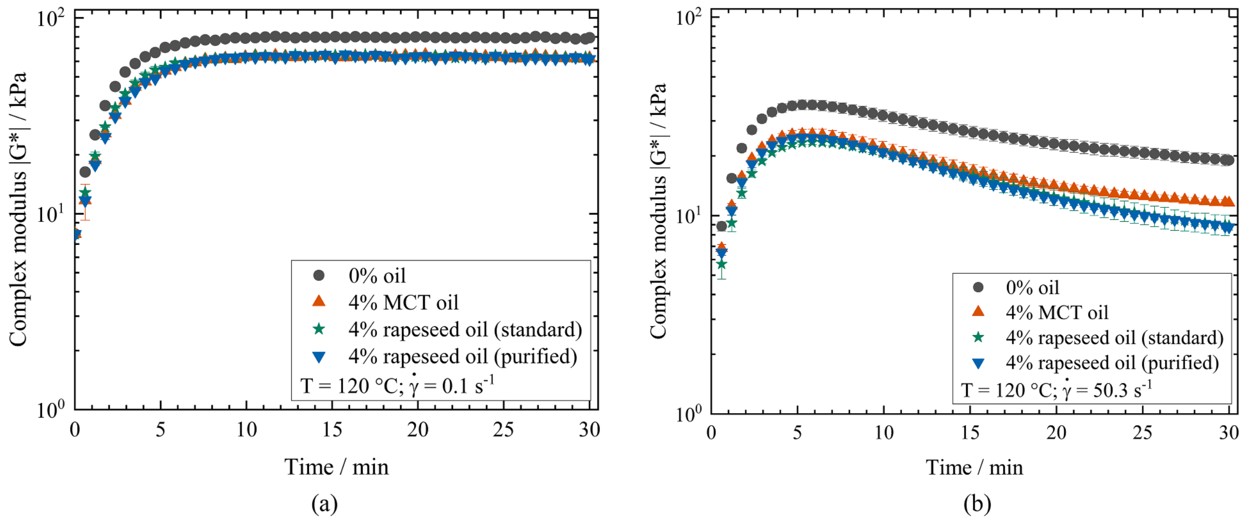

Fig. 4. Change in complex modulus |G*| of wheat gluten as a function of time and different oil types, i.e. MCT oil, rapeseed oil (standard), rapeseed oil (purified), during (a) thermal treatment (temperature: 120 °C; strain: 0.98%; frequency: 1.0 Hz) and (b) thermomechanical treatment (temperature: 120 °C; strain: 80%; frequency: 10 Hz). All doughs are prepared with emulsified oil.

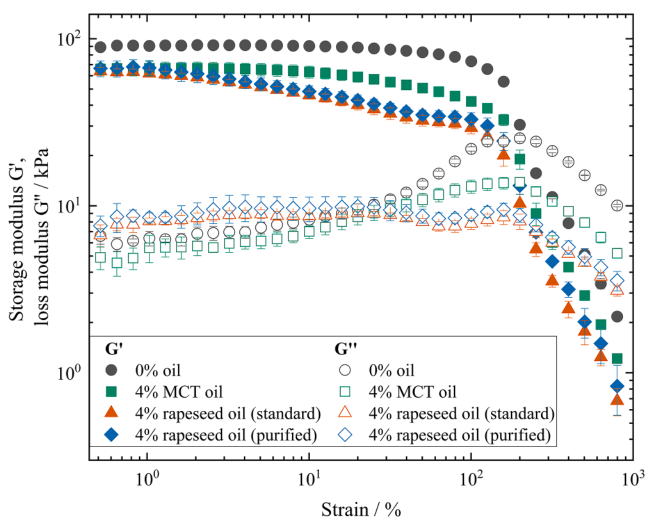

Fig. 5. Strain sweeps of wheat gluten doughs containing 0% oil, 4% MCT oil, 4% rapeseed oil (standard) and 4% rapeseed oil (purified). The temperature was kept constant at 65 °C and the frequency was set to 1.0 Hz.

can still be seen, for the 4% rapeseed oil samples (standard and purified) almost no strain overshoot occurs.

3.4. Visualization of the oil phase embedded in the gluten network

It is known from literature that oil forms a dispersed phase within a gluten network (Gwiazda et al., 1987; Kendler et al., 2021). The size distribution of this dispersed phase could have an impact on the rheological behavior of wheat gluten. In order to visualize the oil phase, we analyzed the different samples from the previous chapters via CLSM imaging.

3.4.1. Influence of oil concentration and oil addition method

First, we took CLSM images of the doughs before treatment in the closed cavity rheometer. In Fig. 6, CLSM images of untreated wheat gluten doughs with 2%, 4% and 6% MCT oil for both different oil addition methods (non-emulsified/emulsified) are depicted. The gluten matrix appears in green, while the oil droplets appear in red. For both oil addition methods a slight increase in oil droplet size with increasing oil content can be observed. An increasing droplet size as the oil concentration increases, is known from previous studies and can be explained by reduced droplet break-up as well as increased droplet coalescence during the mixing process of the doughs (Opaluwa et al., 2023). However, for doughs, where non-emulsified oil was added, the increase in droplet size with increasing oil content is much more pronounced compared to the doughs with emulsified oil.

Comparing the doughs with non-emulsified and emulsified oil, the droplets of the doughs with non-emulsified oil appear remarkably bigger at all oil contents. Moreover, the oil droplets seem to be less embedded and incorporated into the protein matrix. For example, in the CLSM image of 4% MCT oil (non-emulsified) oil droplets appear to be squeezed into a gap of the wheat gluten matrix (oil droplets are highlighted with yellow mark). Overall, the CLSM images show that oil that is added in form of emulsions is dispersed into smaller droplets with a more homogeneous droplet size, while non-emulsified oil forms bigger oil droplets, which are less incorporated into the gluten matrix. This indicates that the pre-emulsified oil droplets are more stable against oil droplet coalescence.

3.4.2. Influence of thermal and thermomechanical treatment

In the next step, we analyzed the oil droplets in the wheat gluten sample after thermal treatment (120 °C, 0.1 s^{-1}, 30 min) and after thermomechanical treatment (120 °C, 50.3 s^{-1}, 30 min) in the closed cavity rheometer. The CLSM images of the samples that contained 4% MCT oil (non-emulsified/emulsified) and 4% rapeseed oil (standard/purified) are shown in Fig. 7. It is evident that the appearance of the wheat gluten matrix is changed by both thermal and thermomechanical treatment. After treatment, the network appears to be more compact, and elongated voids can be observed.

First, we want to highlight the differences between the 4% MCT oil doughs containing non-emulsified and emulsified oil. In the CLSM images of the samples containing emulsified MCT oil (Fig. 7, second row), the size of the oil droplets does not seem to change noticeably by either thermal or thermomechanical treatment. Most oil droplets are embedded in and deformed along the protein matrix. Only a few oil droplets exhibit an almost spherical shape and are located in the voids of the gluten matrix (highlighted in yellow). Looking at the CLSM images of the samples with 4% non-emulsified MCT oil (Fig. 7, first row) some differences can be observed. First, the oil droplets are remarkably bigger than the oil droplets in the emulsified samples, which was already addressed in Section 3.4.1. Secondly, the localization of the oil droplets

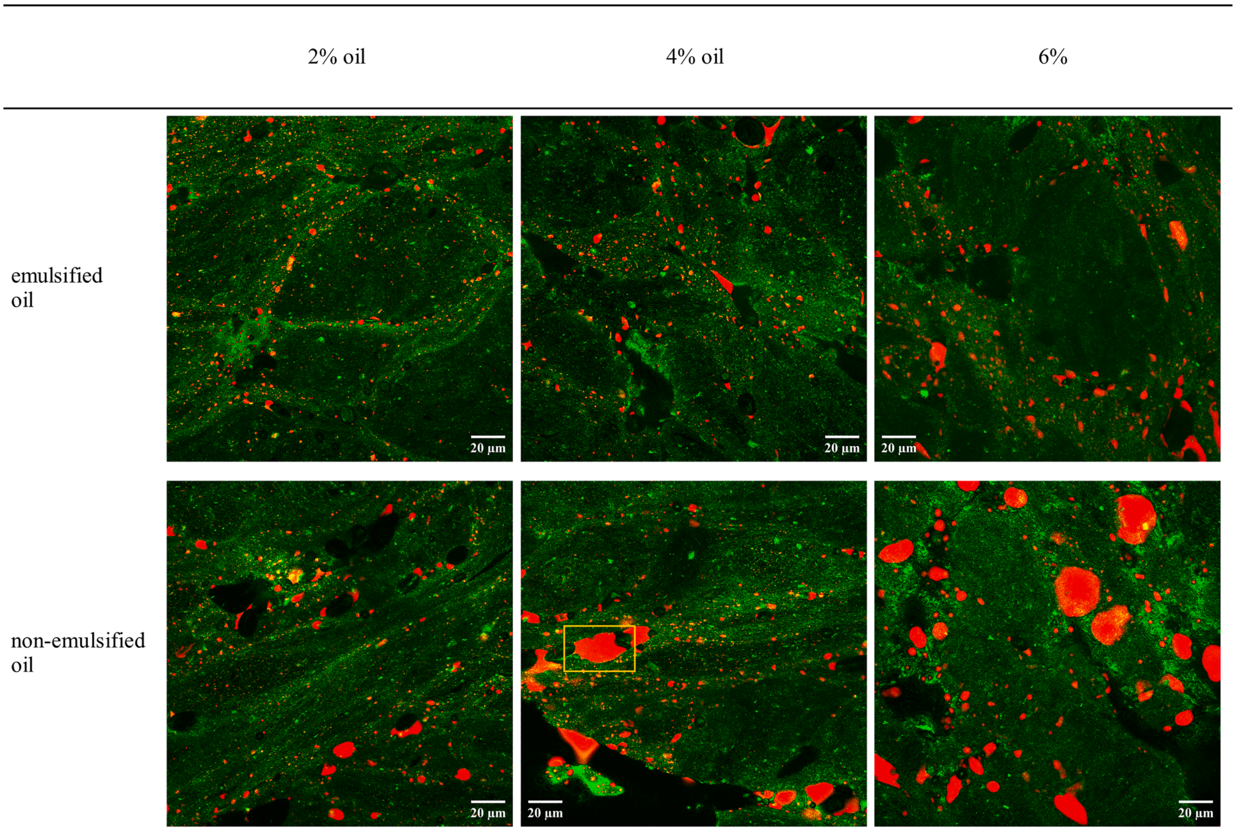

Fig. 6. CLSM images of untreated doughs containing 2%, 4% and 6% MCT oil (non-emulsified/emulsified), respectively. Protein matrix appears in green, oil phase appears in red.

in the network as well as the shape of the oil droplets look different. A majority of the oil droplets are not incorporated into the network, but are located in the voids of the wheat gluten matrix. This impression is supported by the shape of the droplets, which is less deformed along the protein matrix and more spherical. In particular, in the sample subjected to thermomechanical treatment, a large number of spherical droplets are found (highlighted in yellow). It could be that due to the polymerization of the wheat gluten and the associated increase in |G*|, oil is displaced from the matrix to some extent when subjected to mechanical stresses.

Finally, we want to compare CLSM images of samples containing different oil types. Fig. 7 (row 3–4) shows CLSM images of samples containing 4% rapeseed oil (standard) and 4% rapeseed oil (purified), respectively. Both samples are prepared from oil-in-water emulsions. Between the samples containing different oil types no remarkable difference neither in droplet size nor in droplet shape can be observed. As with the 4% emulsified MCT sample (row 2), the oil in the 4% emulsified rapeseed oil samples (standard/emulsified) appears homogeneously dispersed and embedded in the wheat gluten matrix. These results indicate that different oil types, and thus different interfacial tensions between the oil and the aqueous protein phase, do not affect the dispersion of the oil in the protein matrix resulting in similar oil droplet size distributions.

4. Discussion

4.1. Dilution effect vs plasticizing effect

In our study, we used a rheological approach to measure the impact of different oil-related influence parameters on the protein matrix as we believe a relevant impact on the extrusion process will only occur if changes in rheological properties can be detected. Our results show that the complex modulus |G*| decreases with increasing oil concentration. This was shown for different oil addition methods as well as for both thermal and thermomechanical treatment (see Fig. 1 and Fig. 2). A reduction in viscosity when oil is added to concentrated biopolymers is well-known (Opaluwa et al., 2023; Saavedra Isusi et al., 2023b; Wang et al., 2022a). Different explanations therefore can be found in literature. One of the most accepted hypothesis is that oil acts as a plasticizer (Fu et al., 1997; Ilo et al., 2000). In the introduction, we already summarized different theories about how plasticizers function. Generally, it is expected that a plasticizer reduces the viscosity by increasing the free volume between protein molecules and thus reducing the entanglement density. According to plasticizer theories, the properties of the plasticizer molecules (size, compatibility) largely determine how pronounced the resulting plasticizing effect is.

To compare the effect of oil and water, we prepared three different samples. One sample with a lower plasticizer concentration of 38.8 wt% (only water) and three samples with a higher plasticizer concentration (only water or oil/water mixture) of 42.4 wt%. For clarification, the compositions are listed in Table A3 (Appendix). Since both water molecules and triglyceride molecules differ greatly in their size and compatibility with protein molecules, we would expect a differently pronounced plasticizing effect and thus differences in rheological behavior. However, Fig. 2(a) shows that |G*| of the 0% oil + 6% water dough and the 6% oil doughs (non-emulsified/emulsified) do not differ despite their different plasticizer composition. This indicates that the decrease in |G*| with increasing plasticizer concentration from 38.8 wt % to 42.4 wt% is due to the decrease in protein concentration per unit volume, which is more likely a dilution effect rather than a plasticizing effect in itself. The results from Fig. 4(a), in which MCT oil is compared with rapeseed oil (standard/purified), support this suggestion. In this case, again, no difference in |G*| can be observed between the different oil types, although the different oil types are composed of different triglyceride molecules with different properties in terms of size, purity and

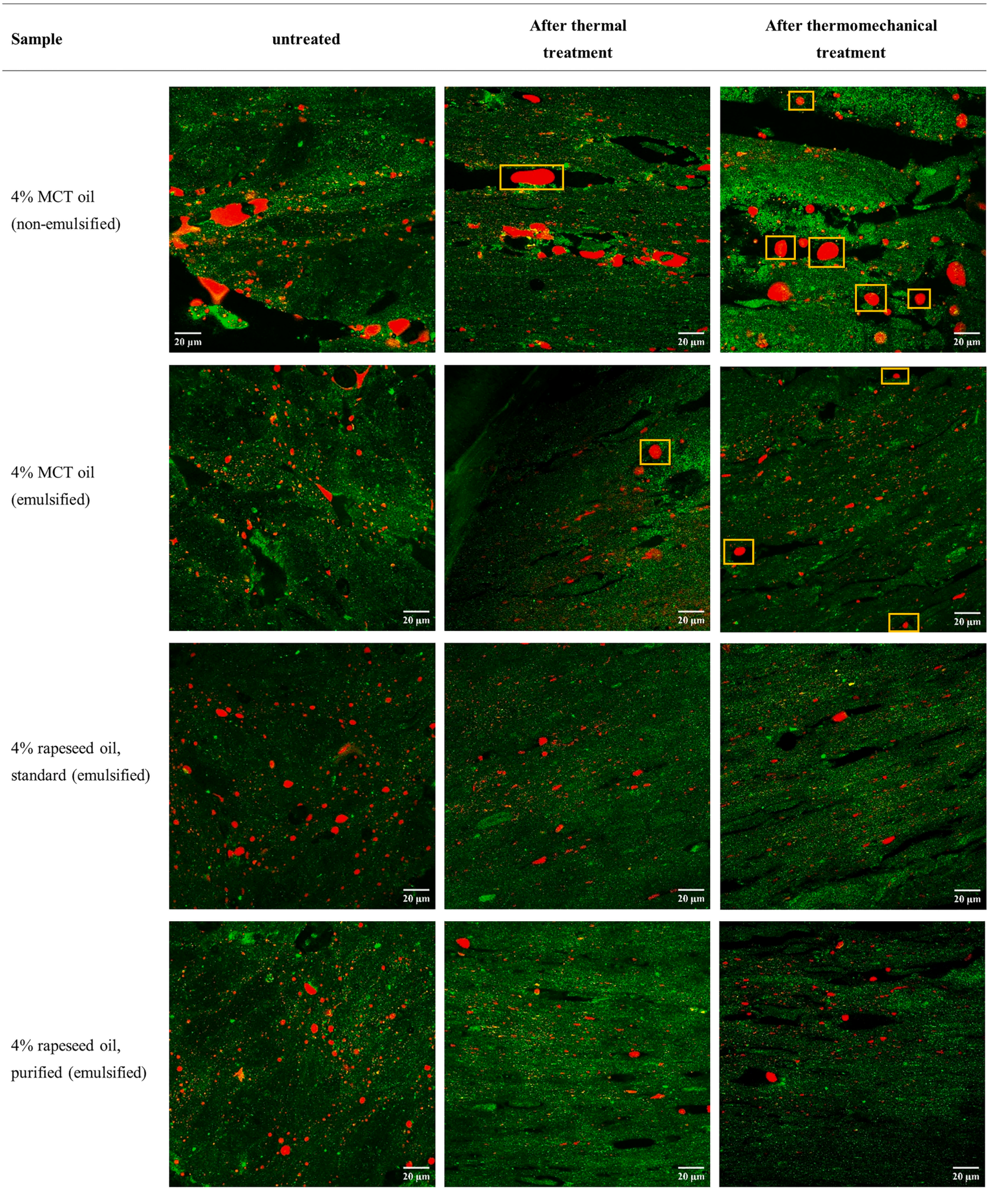

Fig. 7. CLSM images of untreated samples, samples after thermal treatment and after thermomechanical treatment of doughs containing 4% MCT oil (non-emulsified/emulsified) and 4% emulsified rapeseed oil (standard/purified), respectively. Protein matrix appears in green, oil phase appears in red.

compatibility.

4.2. *Plasticizing effect vs filler effect*

It is known that in extrusion applications, where high temperatures and high shear stresses prevail at the same time, the influence of oil on the process, often described as lubricating effect, is much greater than that of water in terms of their active concentrations (Guy, 1994; Ilo et al., 2000). In order to investigate this, we performed rheological measurements at extrusion-relevant conditions (120 °C, 50.3 s^{-1}). We found that under shear conditions, there is indeed a large difference between the 0% oil + 6% water sample and the oil-containing samples (see Fig. 2, b). In fact, the maximum $|G^*|$ of the 6% oil (non-emulsified) sample is 70% lower than the maximum $|G^*|$ of the 0% oil + 6% water sample. In addition, strain sweep analysis (see Fig. 3) revealed that the LVE region of doughs with 0% oil + 6% water is longer compared to the LVE region of the two oil-containing samples, meaning that the network without oil is more elastic and more resistant to deformation. The results indicate that under shear conditions the aforementioned dilution effect is enhanced by another effect. By CLSM imaging, we could show that the oil phase is assembled into droplets within the protein matrix (see Fig. 6). Thus, in contrast to water, oil does not act as a plasticizer by occupying the free volume of the protein chains, but rather as a filler by forming droplets within the aqueous protein matrix. It is known from emulsion-filled gels, such as cheese or yoghurt (Barden et al., 2015; Saavedra Isusi et al., 2023a), that the matrix strength is remarkably influenced by the concentration and the size of the oil droplets which are embedded in the matrix as fillers. Under shear conditions, these fillers act as structure-breakers and stress concentration nuclei, leading to a weakening of the protein matrix (Sala et al., 2007). This could explain why the 0% oil + 6% water sample, which does not contain such structure-breakers, is more resistant to deformation. Based on our results, we propose to describe the effect of oil during extrusion as a "filler effect" rather than a "plasticizing effect", although this term is commonly used in the extrusion literature.

Following the literature on emulsion-filled gels, we hypothesized that the size of the oil droplets would have a significant effect on the strength of a highly concentrated gluten matrix formed during thermomechanical treatment. In order to prove this hypothesis, we compared two different sets of doughs with the same oil concentrations but different oil droplet sizes. CLSM images confirmed that the doughs with non-emulsified oil contained bigger oil droplets than the doughs with emulsified oil (see Fig. 6 and Fig. 7). With this experimental design we were able to show that different oil droplet sizes have an effect on the rheological properties. We found that $|G^*|$ of doughs with bigger oil droplets (non-emulsified oil) were significantly lower ($p > 0.05$) than $|G^*|$ of doughs with smaller oil droplets (emulsified oil) at all oil concentration levels (see Fig. 2(b) and Appendix Table A1). As oil droplets lead to a local dilution and thus a local viscosity decrease within the protein network, bigger oil droplets lower the viscosity to a greater extent compared to smaller oil droplets. Moreover, bigger oil droplets cause bigger defects in the protein network, making the network less stable against deformation.

When comparing the doughs containing different oil types, i.e. MCT oil, rapeseed oil (standard) and rapeseed oil (purified), we observed that they all had similar rheological behavior at the same oil concentrations (see Fig. 4), even though the different oil types differ greatly in chemical structure, purity and interfacial tension against water. The CLSM images showed that the oil droplet size was in a comparable size range for all samples (see Fig. 7). These results suggest that the size distribution of the oil droplets is more decisive for the strength of the protein network than the oil type.

4.3. *Oil effect on wheat gluten polymerization*

Lastly, we want to discuss the effect of oil on the reaction behavior of wheat gluten. We observed that for the samples with emulsified and non-emulsified oil, not only the absolute values of the maximum $|G^*|$ differ from each other but also the time at which these maxima are reached (see Fig. 2, b). While for the non-emulsified oil samples the time at which the maximum $|G^*|$ is reached decreases significantly ($p > 0.05$) with increasing oil content, this correlation was not found for the emulsified oil samples (Appendix Table A1 and Table A2). A possible explanation is that as an increase in oil droplet size (non-emulsified oil) results in a decrease in $|G^*|$, the molecular mobility is also increased, leading to a higher probability of crosslinking with other unfolded protein molecules in a shorter time. However, further systematic studies of reaction kinetics and protein-protein and protein-lipid interactions are required to evaluate the effect of oil on the reaction behavior of wheat gluten.

5. Conclusion

In this study, we investigated the effect of oil on the rheological properties of highly concentrated wheat gluten at extrusion-relevant conditions. We used an offline approach that combined rheological measurements in a closed cavity rheometer with CLSM imaging to visualize the oil droplets in the protein matrix before and after the rheological measurements. First, we showed that under extrusion-relevant conditions (high temperature and high shear), the effect of oil addition on the complex modulus $|G^*|$ is much greater than the effect of water addition at the same concentrations. Interestingly, this effect was not significant when no shear was applied. Therefore, we hypothesize that the decrease in $|G^*|$ without shear is due to a dilution effect, while with shear the effect of oil droplets is due to a filler effect. This implies that the oil droplets weaken the protein matrix during deformation, resulting in a remarkable decrease in $|G^*|$.

Second, we found that there is a significant difference between samples containing non-emulsified oil and emulsified oil. Although in both cases the complex modulus $|G^*|$ decreased with increasing oil concentration (0% to 6% oil), this effect was much more pronounced for samples with non-emulsified oil. Since CLSM images showed that the oil droplets are considerably larger for non-emulsified oil than for emulsified oil droplets, we contribute the observed differences in $|G^*|$ to the oil droplet size. We also showed that the oil type and oil purity do neither affect the complex modulus $|G^*|$ nor the size of the oil droplets.

In summary, the results of this study suggest that the oil droplet size has a great influence on the rheological properties of highly concentrated protein matrices under extrusion-relevant conditions, while the oil type has no relevant influence for the oil types investigated. It is necessary to investigate in further studies to what extent the results of this study can be applied to extrusion processes. However, based on our results, we would suggest that the oil droplet size distribution must be controlled in order to control the rheological properties during extrusion processing, even at low oil concentrations.

Ethical statement

Hereby, I, Christina Opaluwa, consciously assure that for the manuscript 'Effect of oil on the rheological properties and reaction behavior of highly concentrated wheat gluten under conditions relevant to high moisture extrusion' the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publication elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
5) The results are appropriately placed in the context of prior and existing research.
6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

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Authors and in the Ethical Statement.

CRediT authorship contribution statement

Christina Opaluwa: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft. **Sarah Deskovski:** Data curation, Investigation. **Heike P. Karbstein:** Resources, Writing – review & editing. **M.Azad Emin:** Conceptualization, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Funding

This work was supported by the German Ministry of Economics and Climate Action (via AiF) and FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn) in the scope of project AiF 20250N.

Acknowledgments

The authors would like to express their thank to Martin Bastmeyer, Marc Hippler and Lukas Lachenmaier from the Department of Cell- and Neurobiology, Zoological Institute, Karlsruhe Institute of Technology for their support with the CLSM Imaging and, to Kerstin Sauther, Lydia Schütz and Corinna Raith for the experimental support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100307.

References

Akdogan, H., 1999. High moisture food extrusion. Int. J. Food Sci. Technol. 34 (3), 195–207. https://doi.org/10.1046/j.1365-2621.1999.00256.x.

Arêas, J.A, 1992. Extrusion of food proteins. Crit. Rev. Food Sci. Nutr. 32 (4), 365–392. https://doi.org/10.1080/10408399209527604.

Barden, L.M., Osborne, J.A., McMahon, D.J., Foegeding, E.A, 2015. Investigating the filled gel model in Cheddar cheese through use of Sephadex beads. J. Dairy Sci. 98 (3), 1502–1516. https://doi.org/10.3168/jds.2014-8597.

Chen, J., Dickinson, E., 1999. Effect of surface character of filler particles on rheology of heat-set whey protein emulsion gels. Colloid. Surf. B: Biointerfaces 12 (3–6), 373–381. https://doi.org/10.1016/S0927-7765(98)00091-5.

Chen, Y., Liang, Y., Jia, F., Chen, Di, Zhang, X., Wang, Q., et al., 2020. Effect of extrusion temperature on the protein aggregation of wheat gluten with the addition of peanut oil during extrusion. Int. J. Biol. Macromol. https://doi.org/10.1016/j.ijbiomac.2020.11.017.

Di Gioia, L., Guilbert, S., 1999. Corn protein-based thermoplastic resins: effect of some polar and amphiphilic plasticizers. J. Agric. Food Chem. 47 (3), 1254–1261. https://doi.org/10.1021/jf980976j.

Dille, M.J., Draget, K.I., Hattrem, M.N, 2015. 9 - The effect of filler particles on the texture of food gels. In: Chen, J, Rosenthal, A (Eds.), Modifying Food Texture Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, pp. 183–200. doi:10.1016/B978-1-78242-333-1.00009-7.

Dopierala, K., Javadi, A., Krägel, J., Schano, K.-H., Kalogianni, E.P., Leser, M.E., et al., 2011. Dynamic interfacial tensions of dietary oils. Colloid. Surf. A: Physicochem. Eng. Aspect. 382 (1–3), 261–265. https://doi.org/10.1016/j.colsurfa.2010.11.027.

Egbert, R., Borders, C., 2006. Achieving success with meat analogs. Food Technol. (60), 28–34.

Emin, M.A., Quevedo, M., Wilhelm, M., Karbstein, H.P., 2017. Analysis of the reaction behavior of highly concentrated plant proteins in extrusion-like conditions. Innovat. Food Sci. Emerg. Technol. 44, 15–20. https://doi.org/10.1016/j.ifset.2017.09.013.

Farjami, T., Madadlou, A., 2019. An overview on preparation of emulsion-filled gels and emulsion particulate gels. Trends Food Sci. Technol. 86, 85–94. https://doi.org/10.1016/j.tifs.2019.02.043.

Fu, J., Mulvaney, S.J., Cohen, C., 1997. Effect of added fat on the rheological properties of wheat flour doughs. Cereal Chem. 74 (3), 304–311. https://doi.org/10.1094/CCHEM.1997.74.3.304.

Geremias-Andrade, I.M., Souki, N.P., Moraes, I.C.F., Pinho, S.C., 2016. Rheology of emulsion-filled gels applied to the development of food materials. Gels 2 (3), 22. https://doi.org/10.3390/gels2030022.

Guy, R.C.E., 1994. Raw materials for extrusion cooking processes. editor. In: Frame, ND (Ed.), The Technology of Extrusion Cooking. Springer US, Boston, MA, s.l., pp. 52–72

Gwiazda, S., Noguchi, A., Saio, K., 1987. Microstructural studies of texturized vegetable protein products: effects of oil addition and transformation of raw materials in various sections of a twin screw extruder. Food Struct. 6 (1), 57–61.

Hernandez-Izquierdo, V.M., Krochta, J.M., 2008. Thermoplastic processing of proteins for film formation—a review. J. Food Sci. 73 (2), R30–R39. https://doi.org/10.1111/j.1750-3841.2007.00636.x.

Högg, E., Rauh, C., 2023. Towards a better understanding of texturization during high-moisture extrusion (HME)—Part I: modeling the texturability of plant-based proteins. Foods 12. https://doi.org/10.3390/foods12101955.

Hyun, K., Wilhelm, M., Klein, C.O., Cho, K.S., Nam, J.G., Ahn, K.H., et al., 2011. A review of nonlinear oscillatory shear tests: analysis and application of large amplitude oscillatory shear (LAOS). Prog. Polym. Sci. 36 (12), 1697–1753. https://doi.org/10.1016/j.progpolymsci.2011.02.002.

Ilo, S., Schoenlechner, R., Berghofe, E., 2000. Role of lipids in the extrusion cooking processes. Grasas y Aceites 51 (1–2), 97–110. https://doi.org/10.3989/GYA.2000.V51.I1-2.410.

Jia, F., Wang, J., Wang, Q., Zhang, X., Chen, Di, Chen, Y., et al., 2020. Effect of extrusion on the polymerization of wheat glutenin and changes in the gluten network. J. Food Sci. Technol. 57 (10), 3814–3822. https://doi.org/10.1007/s13197-020-04413-6.

Kendler, C., Duchardt, A., Karbstein, H.P., Emin, M.A., 2021. Effect of oil content and oil addition point on the extrusion processing of wheat gluten-based meat analogues. Foods. 10 (4), 697. https://doi.org/10.3390/foods10040697.

Kokini, J.L., Cocero, A.M., Madeka, H., de Graaf, E., 1994. The development of state diagrams for cereal proteins. Trends Food Sci. Technol. 5 (9), 281–288. https://doi.org/10.1016/0924-2244(94)90136-8.

Lefèvre, T., Subirade, M., Pézolet, M., 2005. Molecular description of the formation and structure of plasticized globular protein films. Biomacromolecules. 6 (6), 3209–3219. https://doi.org/10.1021/bm050540u.

Leister, N., Karbstein, H.P., 2021. Influence of hydrophilic surfactants on the W1–W2 coalescence in double emulsion systems investigated by single droplet experiments. Colloid. Interfaces 5 (2), 21. https://doi.org/10.3390/colloids5020021.

Liu, K., Hsieh, F.-H., 2008. Protein-protein interactions during high-moisture extrusion for fibrous meat analogues and comparison of protein solubility methods using different solvent systems. J. Agric. Food Chem. 56 (8), 2681–2687. https://doi.org/10.1021/jf073343q.

McClements, D.J., Monahan, F.J., Kinsella, J.E., 1993. Effect of emulsion droplets on the rheology of whey protein isolate gels. J. Texture Stud. 24 (4), 411–422. https://doi.org/10.1111/j.1745-4603.1993.tb00051.x.

Mekonnen, T., Mussone, P., Khalil, H., Bressler, D., 2013. Progress in bio-based plastics and plasticizing modifications. J. Mater. Chem. A 1 (43), 13379–13398. https://doi.org/10.1039/C3TA12555F.

Opaluwa, C., Lott, T., Karbstein, H.P..., Emin, M...A., 2023. Encapsulation of oil in the high moisture extrusion of wheat gluten: interrelation between process parameters, matrix viscosity and oil droplet size. Future Foods. 7, 100222 https://doi.org/10.1016/j.fufo.2023.100222.

Palanisamy, M., Franke, K., Berger, R.G., Heinz, V., Töpfl, S., 2019. High moisture extrusion of lupin protein: influence of extrusion parameters on extruder responses and product properties. J. Sci. Food Agric. 99 (5), 2175–2185. https://doi.org/10.1002/jsfa.9410.

Pietsch, V.L., Bühler, J.M., Karbstein, H.P., Emin, M.A., 2019a. High moisture extrusion of soy protein concentrate: influence of thermomechanical treatment on protein-protein interactions and rheological properties. J. Food Eng. https://doi.org/10.1016/j.jfoodeng.2019.01.001.

Pietsch, V.L., Karbstein, H.P., Emin, M.A., 2018. Kinetics of wheat gluten polymerization at extrusion-like conditions relevant for the production of meat analog products. Food Hydrocoll. 85, 102–109. https://doi.org/10.1016/j.foodhyd.2018.07.008.

Pietsch, V.L., Werner, R., Karbstein, H.P., Emin, M.A., 2019b. High moisture extrusion of wheat gluten: relationship between process parameters, protein polymerization, and final product characteristics. J. Food Eng. 259, 3–11. https://doi.org/10.1016/j.jfoodeng.2019.04.006.

Pommet, M., Morel, M.-H., Redl, A., Guilbert, S., 2004. Aggregation and degradation of plasticized wheat gluten during thermo-mechanical treatments, as monitored by rheological and biochemical changes. Polymer. (Guildf) 45 (20), 6853–6860. https://doi.org/10.1016/j.polymer.2004.07.076.

Rowat, S.J., Legge, R.L., Moresoli, C., 2021. Plant protein in material extrusion 3D printing: formation, plasticization, prospects, and challenges. J. Food Eng. 308, 110623 https://doi.org/10.1016/j.jfoodeng.2021.110623.

Saavedra Isusi, G.I., Marburger, J., Lohner, N., van der Schaaf, Ulrike S., 2023a. Texturing of soy yoghurt alternatives: pectin microgel particles serve as inactive fillers and weaken the soy protein gel structure. Gels. 9 (6), 473. https://doi.org/10.3390/gels9060473.

Saavedra Isusi, G.I., Pietsch, V., Beutler, P., Hoehne, S., Leister, N., 2023b. Influence of rapeseed oil on extruded plant-based meat analogues: assessing mechanical and rheological properties. Processes 11 (7). https://doi.org/10.3390/pr11071871.

Sala, G., van Aken, G.A., Stuart, M.A.C., van de Velde, F., 2007. Effect of droplet–matrix interactions on large deformation properties of emulsion-filled gels. J. Texture Stud. 38 (4), 511–535. https://doi.org/10.1111/j.1745-4603.2007.00110.x.

Schreuders, F.K.G., Sagis, L.M.C., Bodnár, I., Erni, P., Boom, R.M., van der Goot, A.J., 2021. Small and large oscillatory shear properties of concentrated proteins. Food Hydrocoll. 110, 106172 https://doi.org/10.1016/j.foodhyd.2020.106172.

Tirtaatmadja, V., Tam, K.C., Jenkins, R.D., 1997. Superposition of oscillations on steady shear flow as a technique for investigating the structure of associative polymers. Macromolecules. 30 (5), 1426–1433. https://doi.org/10.1021/ma960098v.

van der Sman, R.G.M., van der Goot, A.J., 2023. Hypotheses concerning structuring of extruded meat analogs. Curr. Res. Food Sci. 6 https://doi.org/10.1016/j.crfs.2023.100510.

van Vliet, T., 1988. Rheological properties of filled gels. Influence of filler matrix interaction. Colloid Polym. Sci. 266 (6), 518–524. https://doi.org/10.1007/BF01420762.

Wang, H., Wang, R., Zhang, L., Zhang, W., Bakalis, S., Li, Y., et al., 2023. Physicochemical properties, texture, and in vitro protein digestibility in high-moisture extrudate with different oil/water ratio. Food Res. Int. 163, 112286 https://doi.org/10.1016/j.foodres.2022.112286.

Wang, H., Zhang, L., Czaja, T.P., Bakalis, S., Zhang, W., Lametsch, R., 2022a. Structural characteristics of high-moisture extrudates with oil-in-water emulsions. Food Res. Int. 158, 111554 https://doi.org/10.1016/j.foodres.2022.111554.

Wang, Y., Tuccillo, F., Lampi, A.-M., Knaapila, A., Pulkkinen, M., Kariluoto, S., et al., 2022b. Flavor challenges in extruded plant-based meat alternatives: a review. Compr. Rev. Food Sci. Food Saf. https://doi.org/10.1111/1541-4337.12964.

Wittek, P., Ellwanger, F., Karbstein, H.P., Emin, M.A., 2021a. Morphology development and flow characteristics during high moisture extrusion of a plant-based meat analogue. Foods. 10 (8) https://doi.org/10.3390/foods10081753.

Wittek, P., Walther, G., Karbstein, H.P., Emin, M.A., 2021b. Comparison of the rheological properties of plant proteins from various sources for extrusion applications. Foods. 10 (8), 1700. https://doi.org/10.3390/foods10081700.

Wittek, P., Zeiler, N., Karbstein, H.P., Emin, M.A., 2020. Analysis of the complex rheological properties of highly concentrated proteins with a closed cavity rheometer. Appl. Rheol. 30 (1), 64–76. https://doi.org/10.1515/arh-2020-0107.

Wittek, P., Zeiler, N., Karbstein, H.P., Emin, M.A., 2021c. High moisture extrusion of soy protein: investigations on the formation of anisotropic product structure. Foods. 10 (1) https://doi.org/10.3390/foods10010102.